

# **Monoclonal regulatory T cells provide insights into T cell suppression**

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## Supplementary figure 1

a) Representative flow cytometry plots show CD69 expression on CFSE labelled OT-II Tconvs from suppressive (-----) and non-suppressive (-----) cultures at a  $1_{Tconv}/4_{Treg}$  ratio at 72h. Numbers in the plots depict % of cells in each quadrant, n=3- 5.

b) Representative flow cytometry plots show CD25 expression on CFSE labelled OT-II Tconvs from suppressive (-----) and non-suppressive (-----) cultures at a  $1_{Tconv}/4_{Treg}$  ratio at 72h, n=3-5. Numbers in the plots represent % of cells in each quadrant, n=3-5.

c) Representative histograms show CD44, CD62L, CD69, Tbet, CD5 and CD25 expression on OT-II Tconvs in suppressive (with 3K peptide, -----) vs. non-suppressive (without 3K peptide, -----) co-cultures at a  $1_{Tconv}/4_{Treg}$  ratio at 72h.

d) Inguinal lymph nodes from PBS injected (top) or IL-2 complex (IL-2C) injected (bottom) B3K506 Treg mice.

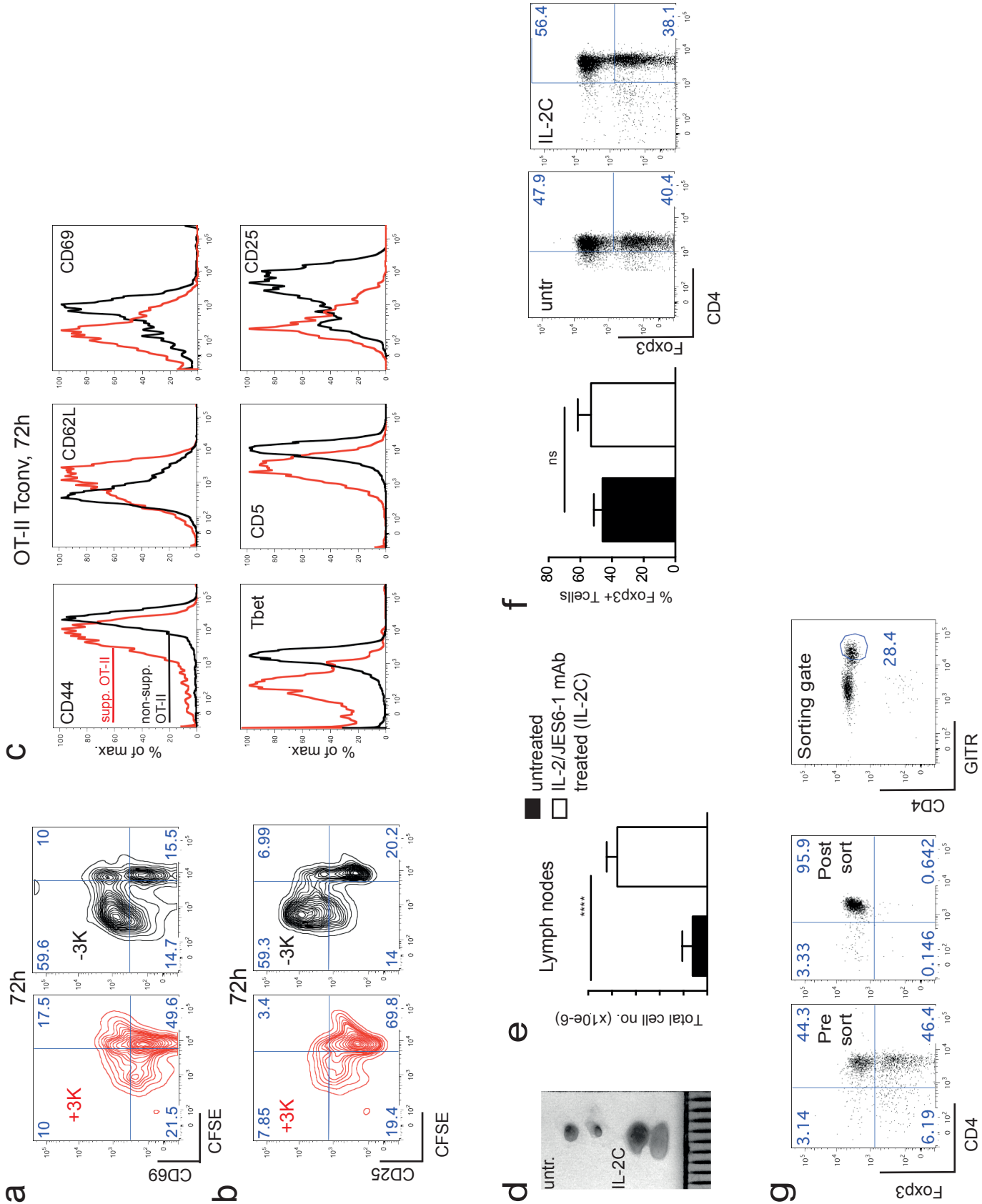
e) Bar graph shows mean number +/- SEM of LN cells isolated from PBS injected (■) or IL-2 complex (IL-2C) injected (□) B3K506 Treg mice, n=3. f) Bar graph and representative flow cytometry plots show mean % +/- SEM of Foxp3+ LN T cells isolated from mice described in D, n=3. Numbers in the plots depict % of cells in quadrant.

g) Representative flow cytometry plots of B3K506 Tregs pre- and post- cell sorting, which was carried out based on CD4 and GITR expression. Numbers in the plots depict (%) of cells in gate/quadrant.

## Supplementary figure 2

- a) Graph shows mean % of CD69<sup>+</sup> B3K506 Tconvs +/-SEM in response to various peptide antigens used at various concentrations. Cells were cultured for 24 hours, n=3.
- b) Bar graph shows mean OT-II proliferation index +/-SD from suppressive co-cultures at a 1<sub>Tconv</sub>/4<sub>Treg</sub> ratio at 72h, where B3K506 Tregs were stimulated with various altered peptide ligands at their EC50 concentration (see A), n=8.
- c) Representative histogram shows CD25 expression on unstimulated B3K506 Tregs with and without addition of exogenous IL-2 (1ng/ml) after 24 hours.
- d) Representative flow cytometry plots show Foxp3 and LAP expression on OT-II Tconvs and B3K506 Tregs from suppressive cultures at a 1<sub>Tconv</sub>/4<sub>Treg</sub> ratio at 72 hours. Numbers in the plots represent % of cells in each gate.
- e) Representative histogram shows CFSE-labelled I-E<sup>d</sup> restricted HA Tconvs in culture with I-A<sup>b</sup> expressing B6 splenic B cells after 72 hours.
- f) In vitro anti-CD3 $\epsilon$  suppression assays with (----) and without (- - -) 50ng/ml recombinant IL-2. Representative proliferation of CFSE labelled polyclonal CD4<sup>+</sup> Tconvs co-cultured with polyclonal B6 Foxp3<sup>EGFP</sup> Tregs (----), monoclonal B3K506 Tregs (- - -) or B3K506 Tconv (---) at a 1<sub>Tconv</sub>/4<sub>Treg</sub> ratio, at 72h.

Supplementary figure 1



Supplementary figure 2

